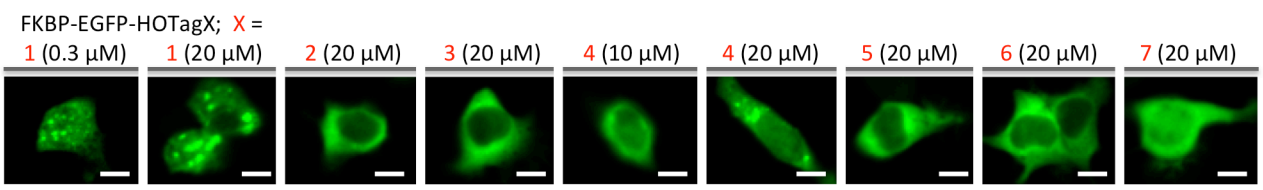
Supplemental Information

Supplemental Figures

A

HOTag1 (pentamer)	TQEDLLKKIMKLLKKQIKLLKKQIKMLKRLEKQ	HOTag5 (tetramer)	AEAESALEYAQQALEKAQLALQAARQALKA
HOTag2 (heptamer)	GEIAQALKEIAKALKEIAWALKEIAQALKG	HOTag6 (tetramer)	TLREIEELLRKIIEDSVRSVAELEDIEKWLKKI
HOTag3 (hexamer)	GEIAKSLKEIAKSLKEIAWSLKEIAKSLKG	HOTag7 (tetramer)	GELAAIKQELAAIKKELAAIKWELAAIKQGAG
HOTag4 (pentamer)	GKIEQILQKIEKILQKIEWILQKIEQILQG		

B

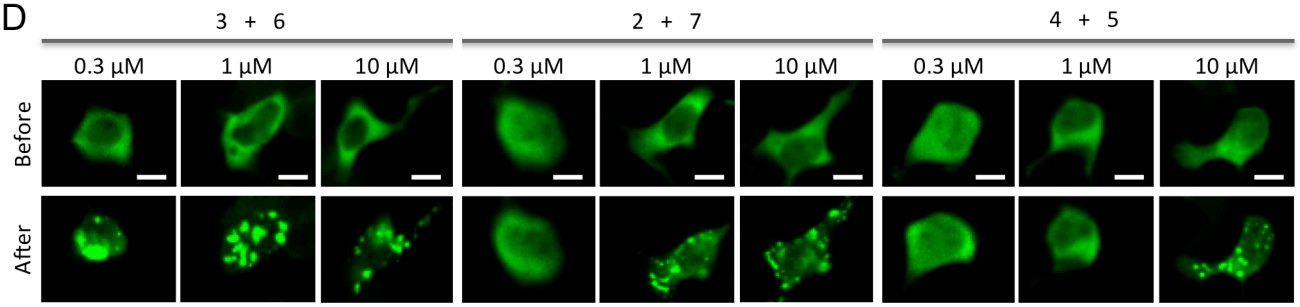


C

FKBP-EGFP-HOTagX + Frb-EGFP-HOTagY

X =	2					3				4			5		6
Y =	3	4	5	6	7	4	5	6	7	5	6	7	6	7	7
Droplet? Rapamycin (−)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Droplet? Rapamycin (+)	+	+	+	+	++	+	+	+++	+	+	+	+	+	+	+

D



E

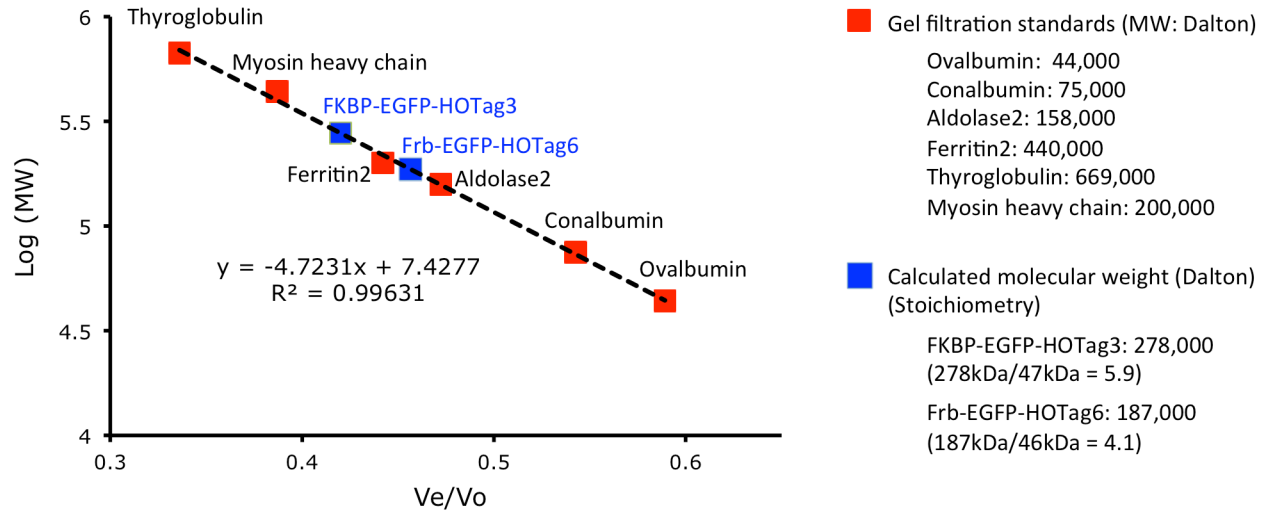


Figure S1. Rational design of inducible protein phase separation by protein-protein interaction.
(A). *De novo* designed coiled coils are used as homo-oligomeric tag (HOTag), which introduces multivalency. (B). HOTags fused to EGFP and FKBP were expressed in HEK293 cells. (C). Protein concentration dependence of inducible droplet formation in HEK293 cells expressing combined HOTag fusions before and after rapamycin addition. −, no droplet. + denotes required protein expression level for droplet formation. +, 10 μM. ++, 1μM. +++, 0.3 μM. (D). Representative images showing EGFP droplet formation in HEK293 cells co-expressing pairs of HOTag fusions upon addition of rapamycin. Images were taken before and after rapamycin addition. (E). Determination of FKBP-EGFP-HOTag3 and Frb-EGFP-HOTag6 stoichiometry using size exclusion chromatography. A standard curve is drawn according to peak elution volume (Ve, elution volume; Vo, column volume) of the gel filtration standards as detected by absorption at 280 nm (red). The unit of MW in Y-axis is Dalton. The measured peak elution of the two fusion proteins are shown in blue. Molecular weight is calculated from the standard curve, which confirms that FKBP-EGFP-HOTag3 and Frb-EGFP-HOTag6 are hexameric and tetrameric, respectively. Scale bar, 10 μm.

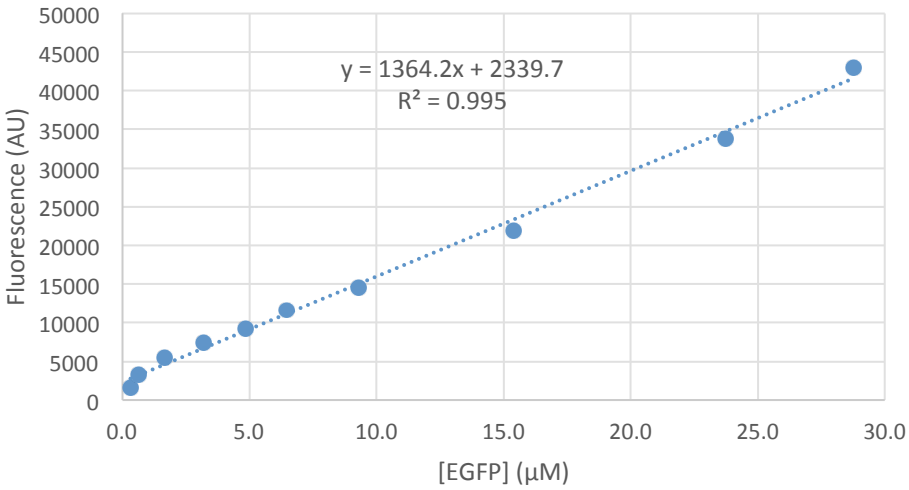


Figure S2. Relationship of EGFP fluorescence brightness and concentration. EGFP was purified and aliquoted at various concentrations. The protein samples were then imaged under the confocal microscope. The fluorescence brightness was recorded, corresponding to EGFP concentration. The plotted line was used to estimate EGFP concentration in living cells based on the fluorescence brightness.

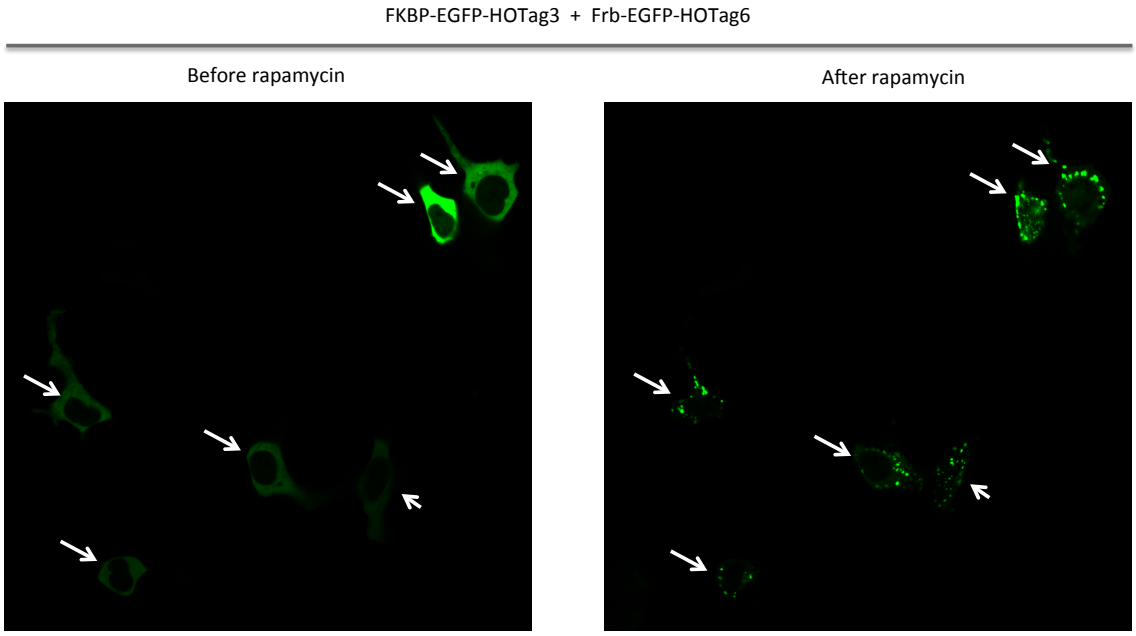


Figure S3. Fluorescence images of HEK293 cells expressing FKBP-EGFP-HOTag3 and Frb-EGFP-HOTag6 upon addition of rapamycin. Arrows point to individual cells.

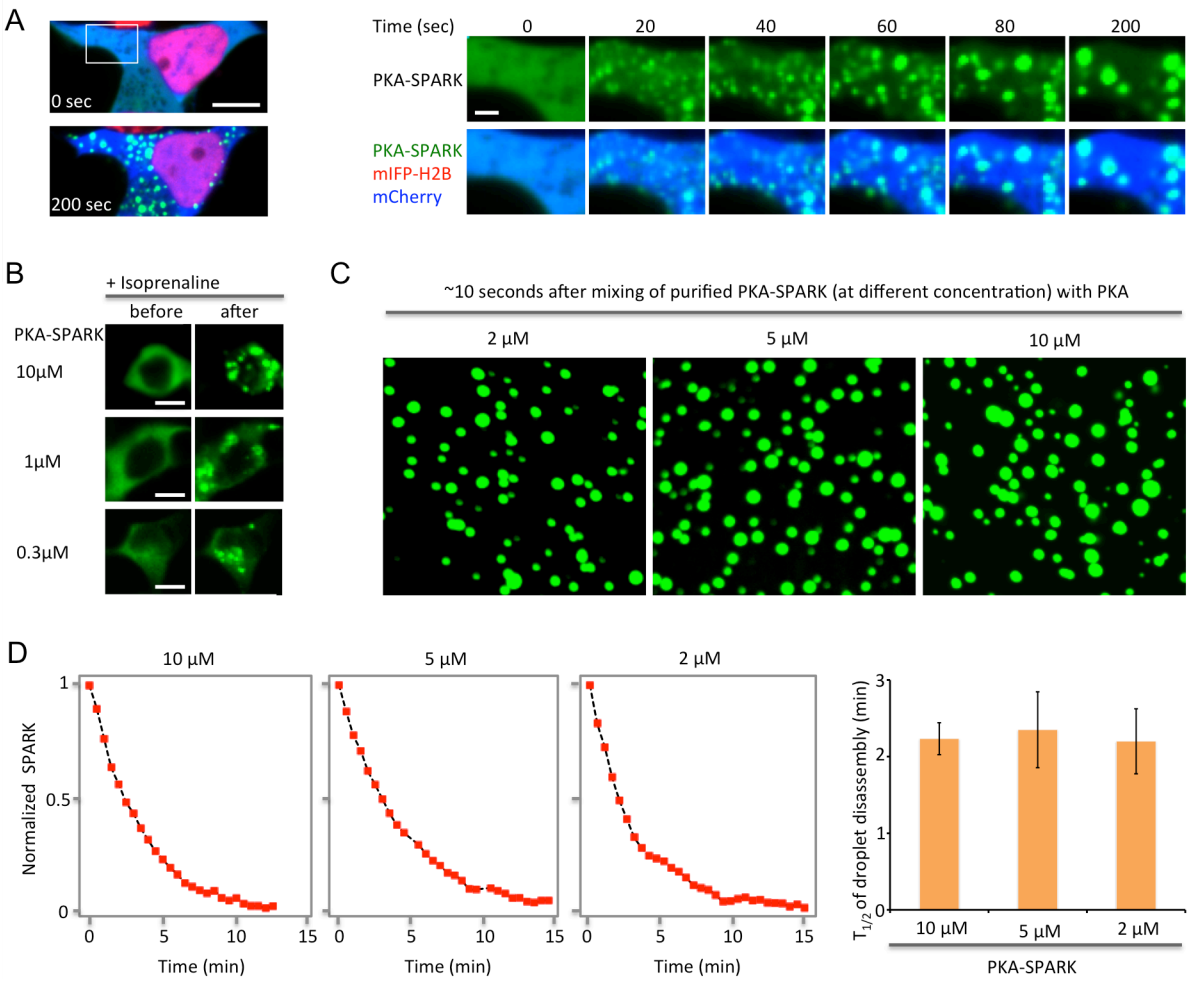


Figure S4. Characterization of PKA-SPARK in HEK293 cells. (A). Time-lapse images of cells stimulated with isoprenaline. Cells expressing PKA-SPARK were also labeled with mCherry and a nucleus located monomeric infrared fluorescent protein (mIFP) fused to histone 2B (H2B). (B). Fluorescence images of cells expressing PKA-SPARK at various concentration. (C). Fluorescence images of purified PKA-SPARK mixed with PKA. Concentration dependence of PKA-SPARK droplet formation upon kinase activation was investigated. Images were taken immediately (~10 seconds) after mixing the purified PKA-SPARK (at specified concentration) with PKA (New England Biolabs). (D). Kinetics of PKA-SPARK droplet disassembly upon de-phosphorylation. Purified PKA-SPARK at specified concentration was pre-incubated with PKA to form droplets. Lambda protein phosphatase (New England Biolabs) was then added to the pre-formed PKA-SPARK droplets. The SPARK signal was calculated as described in the main text. The half time of droplet disassembly was plotted against the concentration of PKA-SPARK (right panel). Standard deviation was obtained from 3 independent measurements. Scale bar, A - B: 10 μm; right panel in A: 2 μm.

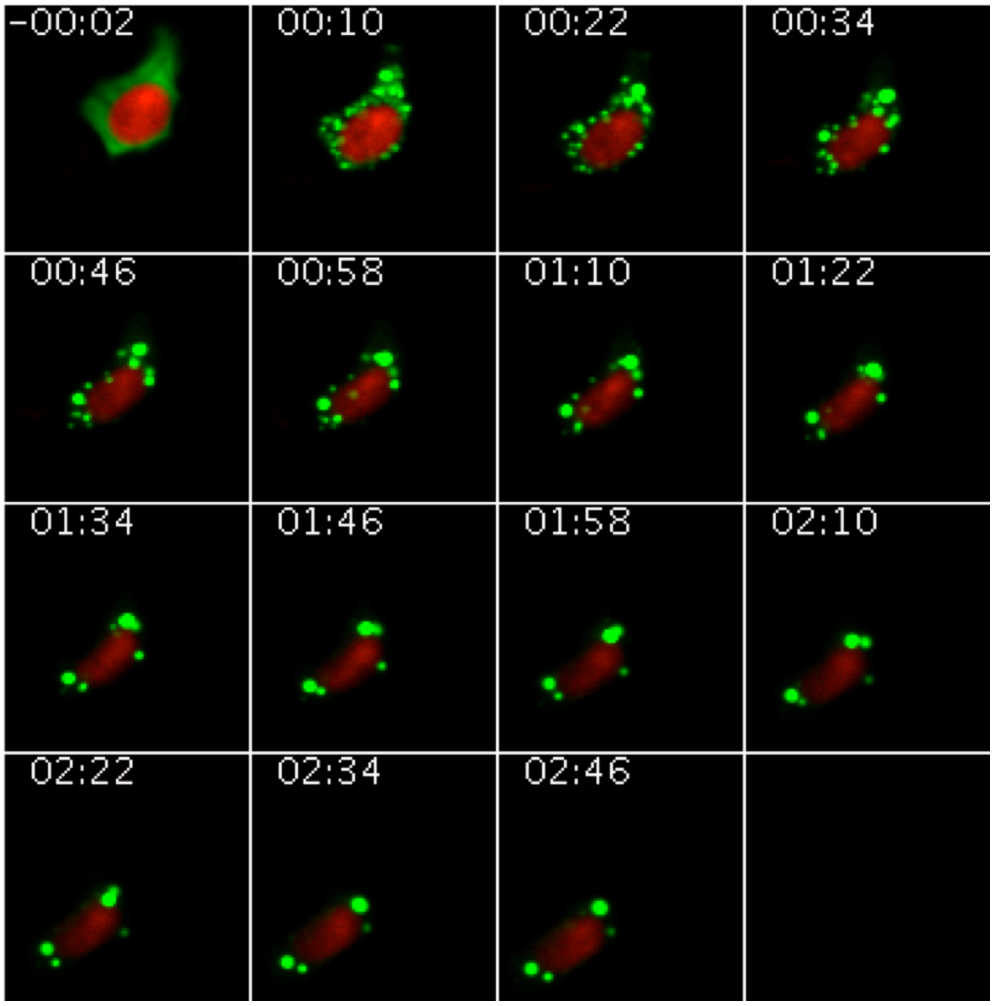


Figure S5. Time-lapse fluorescence images of the cell expressing PKA-SPARK upon addition of isoprenaline. Time is shown in hour:minute. See also Movie S3.

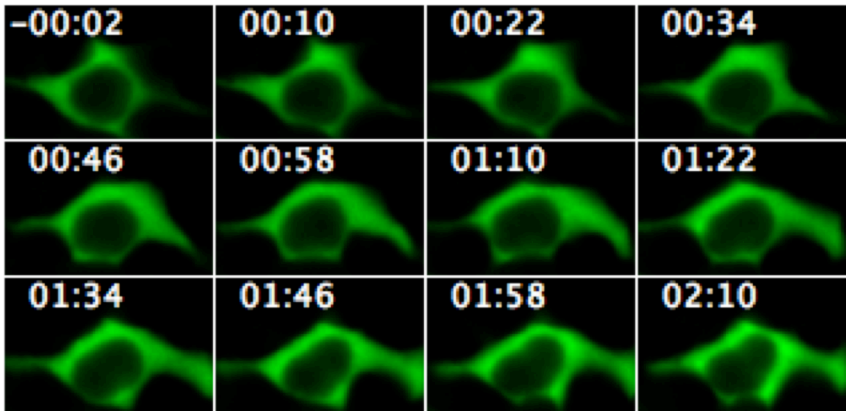


Figure S6. Time-lapse fluorescence images of the PKA-SPARK expressing cell pre-incubated with carvedilol upon addition of isoprenaline. Time is shown in hour:minute.

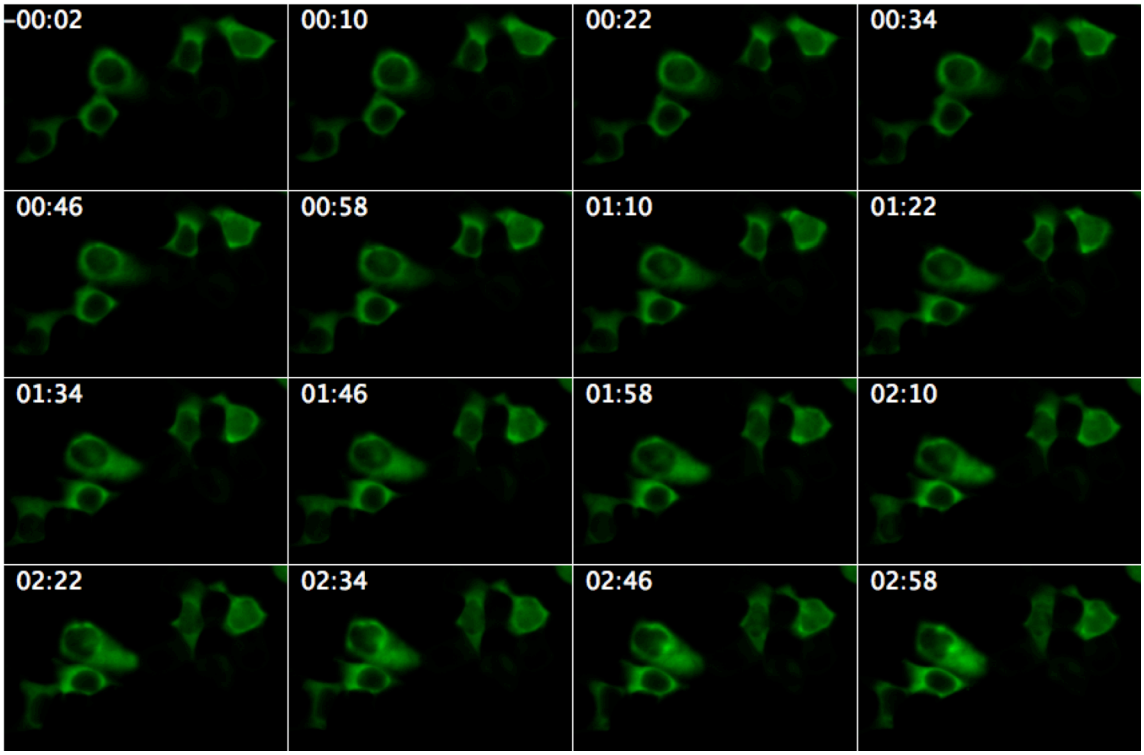


Figure S7. Time-lapse fluorescence images of HEK293 cells expressing PKA-SPARK pre-incubated with caffeine upon addition of adenosine. Time is shown in hour:minute.

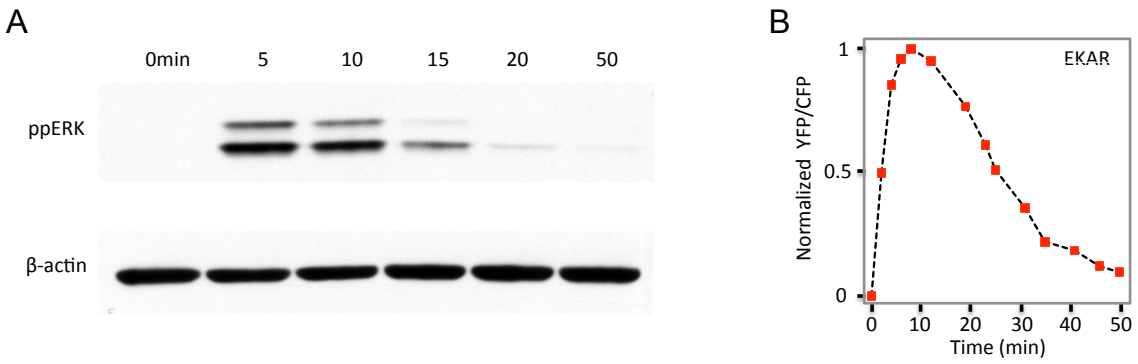


Figure S8. Characterization of ERK activation upon EGF stimulation. (A). Western blot of ppERK upon EGF stimulation of HEK293 cells, with b-actin as a loading control. (B). Time-dependent fluorescence ratio change of the FRET ERK reporter EKAR upon addition of EGF in HEK293 cells. The normalized ratio of yellow fluorescence of YFP (yellow fluorescent protein) over cyan fluorescence of CFP (cyan fluorescent protein) was plotted against time.

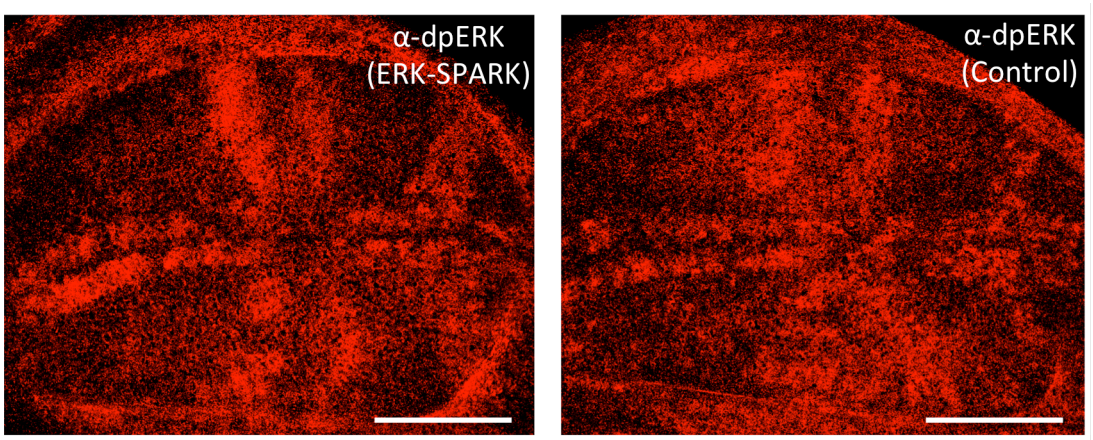


Figure S9. Antibody staining of dpERK in the wing disc in *Drosophila*. Left, dpERK staining of a wing disc expressing ERK-SPARK. Right, dpERK staining of a control wing disc.

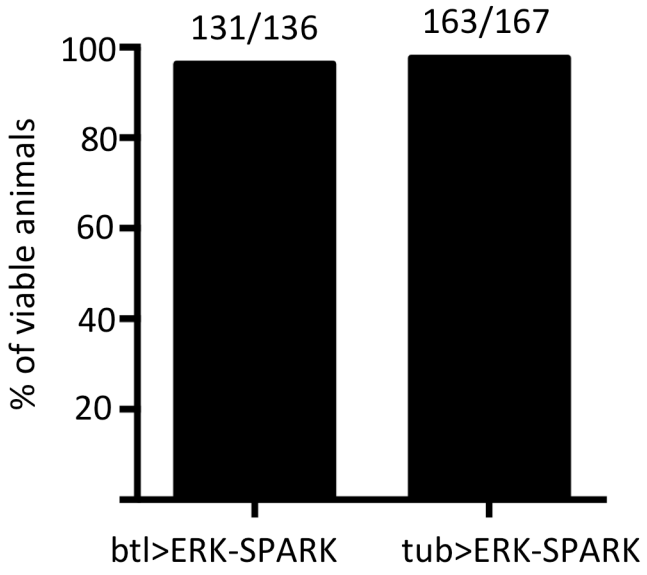


Figure S10. Viability of Drosophila expressing ERK-SPARK. ERK-SPARK was expressed in trachea (btl>ERK-SPARK), or ubiquitously in whole animals (tub>ERK-SPARK). The bar graph represents the number of flies expressing Erk-SPARK over their siblings that possessed balancers. The ratio of animals expressing Erk-SPARK in the trachea (btl-Gal4/+; UAS-Erk-SPARK/+) versus control siblings (btl-Gal4/+; +/TM6B) is 131/136. The ratio of animals ubiquitously expressing Erk-SPARK (tub-Gal4/Erk-HT) versus control siblings (Erk-SPARK/TM3) is 163/167. The survival ratios respect Mendelian distribution.

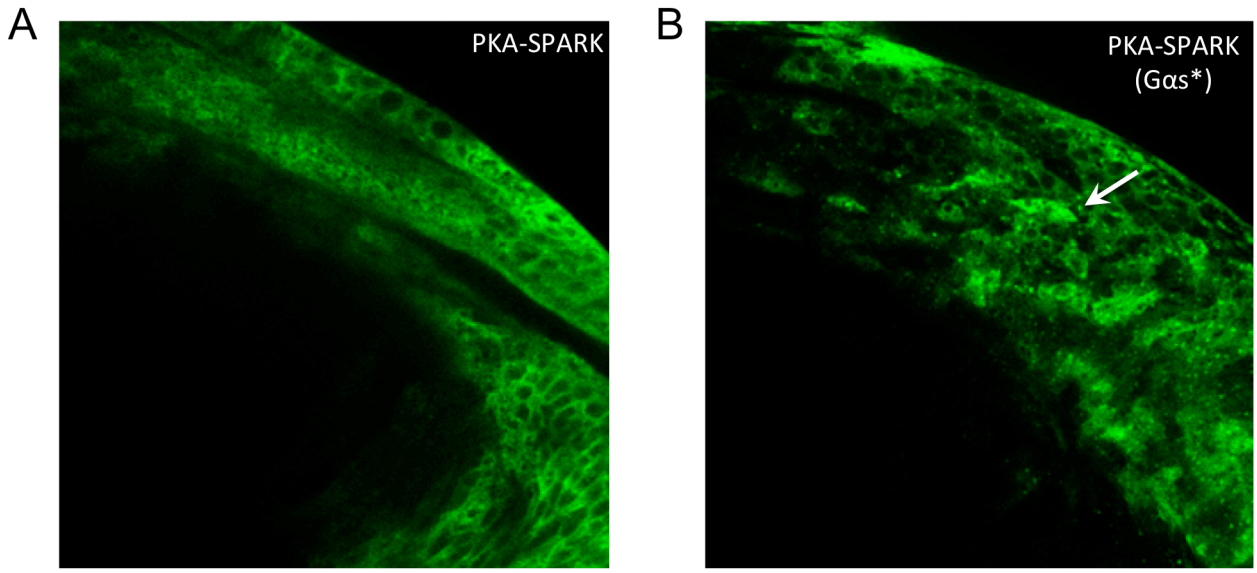


Figure S11. Fluorescence images of PKA-SPARK expressing cells in the wing imaginal disc in Drosophila. (**A**) Wild type wing imaginal disc. (**B**) Wing imaginal disc expressing a constitutively active form of a G protein (Gαs*).

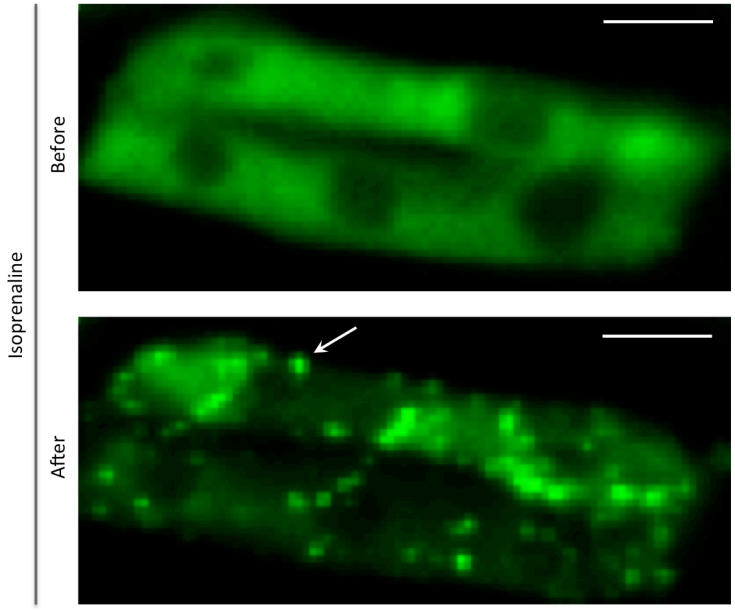


Figure S12. Demonstration of PKA-SPARK in zebrafish. The plasmid encoding PKA-SPARK was injected into zebrafish embryo. The skeletal muscle cells were imaged in the live embryo before and 30 minutes after addition of isoprenaline. Arrow points to EGFP droplet. Scale bar, 10 μm.

Movie S1. Rapamycin-inducible EGFP phase separation. The HEK293 cells expressed FKBP-EGFP-HOTag3 and Frb-EGFP-HOTag6. Time is shown in minutes.

Movie S2. Fluorescence imaging of HEK293 cells expressing PKA-SPARK upon addition of isoprenaline. The cells also expressed mCherry and a nuclear located monomeric infrared fluorescent protein (mIFP). Left, PKA-SPARK (psedocolored green). Middle, mCherry (psedocolored blue) and mIFP-H2B (psedocolored red). Right, merged. Time is shown in minute:second.

Movie S3. Comparison of PKA-SPARK and PKA-KTR. HEK293 cells expressed PKA-SPARK (left) and PKA-KTR (right). Time-lapse imaging was conducted at 10 seconds per frame for PKA-SPARK, and 1 minute per frame for PKA-KTR. The cells were stimulated with isoprenaline to activate PKA.

Movie S4. Time-lapse imaging of the cell expressing PKA-SPARK upon addition of isoprenaline. Time is shown in hour:minute.

Movie S5. Time-lapse fluorescence images of the PKA-SPARK expressing cell pre-incubated with carvedilol upon addition of isoprenaline. Time is shown in hour:minute.

Movie S6. SPARK visualizes dynamic PKA activities in a HEK293 cell stimulated with adenosine. Adenosine activates endogenous adenosine receptors in the HEK293 cell, leading to activation of PKA. Time is shown in hour:minute.

Movie S7. SPARK visualizes dynamic PKA activities in HEK293 cells stimulated with adenosine. Adenosine activates endogenous adenosine receptors in HEK293 cells, leading to activation of PKA. Time is shown in hour:minute.

Movie S8. Time-lapse fluorescence images of HEK293 cells expressing PKA-SPARK pre-incubated with caffeine upon addition of adenosine. Time is shown in hour:minute.

Movie S9. SPARK visualizes transient ERK activation in HEK293 cell. The cell was stimulated with PAR1 agonist SFLLRN, which activates endogenous PAR1. Time is shown in hour:minute.

Movie S10. Z-section of ERK-SPARK expressed in the Drosophila wing imaginal disc. The reporter was driven by Tub-Gal4. Images are shown along Z direction.

Movie S11. SPARK visualizes transient dynamics of ERK activities in vivo. The transgenic Drosophila expressed ERK-SPARK in the trachea. The reporter is expressed by crossing the UAS-ERK-SPARK transgenic line with the btl-GAL4 line.